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INTRODUCTION

In many developing nations around the world, pest birds are a serious threat to human food supplies. In the Sahelian zone of Africa, red-billed quelea (*Quelea quelea)* is of greatest concern. Cereal grain crops within the range of quelea are subject to severe depredations. Therefore, a wide variety of approaches have been taken to alleviate these losses.

In 1972 Magor and Ward reported that during the past 20 years, hundreds of millions of birds have been killed by explosives, flame throwers, and lethal chemicals used to control birds assembled at night in roosts and breeding colonies. Despite this huge control effort, there were no long-term reductions in quelea populations (Crook and Ward, 1968). The high cost, possible contamination, and low successes of such operations led to suggestions for a more ecological approach (Jackson and Park, 1973). Crop protection rather than simple population reduction became the goal (Fumilayo and Akande, 1979; Ward, 1979).

In nature, secondary plant substances (many nonlethal) have protected a wide variety of plant species from vertebrate consumption (Rogers, 1978). For example, astringent tannins in some sorghums successfully deter birds (Harris, 1969). Recent studies at the Denver Wildlife Research Center (DWRC) on bird-resistant sorghum (Bullard et al., 1980; Bullard et al., 1981) led to the consideration of testing commercial tannins for possible utilization as repellents in topical applications to cereal grain crops. The following studies were conducted to determine tannin efficacy in the laboratory.

METHODS AND MATERIALS

The repellent agents employed in cage tests were five water soluble vegetable tannin powders supplied by Tac-Tannins and Chemicals Inc. They were labeled as follows: (1) clarified quebracho extract, (2) wattle extract, (3) chestnut extract, (4) valonea extract, and (5) myrabolam extract. Chemical descriptions of these products are given elsewhere (Bullard and Shumake, 1979).

Tannin powders were surface coated on hulled proso millet with propylene glycol. Weighed amounts of millet (100 g) were placed in a 473 ml glass jar; 0.5 ml of propylene glycol was added and the contents mixed. Then the required amount of wattle tannin was added gradually and stirred, leaving a homogenous coating on the seeds. The control millet was treated only with 0.5 ml propylene glycol.

In the enclosure test, sorghum heads were treated by dipping them in an adhesive solution (0.5% Rhoplex AC-33, Rohm and Haas Chemical Co.) containing 0.5% (~15 kg/ha equivalent) of the wattle-tannin formulation. The control heads were dipped in the adhesive solution only.

Quelea were trapped in Sudan, flown to the DWRC, and held for 90 days' quarantine and acclimation. All birds were held in a large 2.4 X 4.8 X 2.1-m aviary and allowed free access to water, grit, and a maintenance ration of whole grain sorghum, proso millet, and Purina Game Bird Startena.

Cage Tests

The test procedure has been discussed in detail by Bullard and Shumake (1979). One week before any designated test, the birds were transferred from the aviary into 53 X 51 X 41-cm communal cages and then gradually adapted to millet or sorghum test foods by adding them to their maintenance ration in increasing amounts daily. Birds were then transferred to individual cages (constructed by dividing 44 X 25 X 20-cm "double" cages in half with wire mesh) for another two-day pretest adaptation period on the respective test food (Bullard and Shumake, 1979). During this period, birds that consumed more than 3 g of test food were retained for subsequent preference testing.

In all the tests, six naive birds (3 males and 3 females) were utilized. Each of the six birds was given 10 g of the treated and the untreated (control) foods daily for six days. The positions of food cups were alternated daily to eliminate position bias. Spillage was collected in boxes placed under the cages and accounted for in food consumption calculations. Daily food consumption from the control and treated food was recorded for each bird. A preference score was calculated for each bird by using the formula:

$$\text{Percent preference} = \frac{\text{Treated food consumed (g)}}{\text{Treated + control food consumed (g)}} \times 100$$

The percent preference data were treated statistically by analysis of variance. Preferences at the different treatment levels were compared by using the Duncan's Multiple Range Test (MRT. Comparisons between consumption of treated and control foods were done by paired *t*-tests.

Enclosure Test

Again, this test is described in detail in Bullard and Shumake (1979). Ten quelea were housed in a 2.5 X 2.4 X 2.2-m screened cage that allowed them to flock, move freely, and have access to the test foods. In the center, a hexagonally-shaped test apparatus (with 63.5-cm sides) served as a simulated "field" for the placement of sorghum heads. A 26-cm high by 120-cm wide board divided the apparatus into two geometrically identical sections for each of the two test formulations. The control and treated sorghum heads were presented by placing three heads of each on opposite sides of the test apparatus. The stem of a sorghum head was inserted through a 1.2-cm hole drilled in a modified cake pan holder centered 9 cm from the outer edge within each of six segments of test apparatus.

All birds were exposed for six days to the treated and control sorghum heads which were replaced daily. The position of the control and treatment feeder sections was alternated daily to reduce position-habit bias. Food consumption was measured by weighing the air-dried heads before and after each test day. The total difference in weight (corrected for spillage) for the three heads in each section was the amount consumed. A paired *t*-test was used to analyze results.

RESULTS AND DISCUSSION

Test 1 - Screening Commercial Tannin Products for Repellency

In this test, the five commercial products were preference tested at 0.2% on hulled proso millet on quelea in individual cages with the objective being to select one as the candidate repellent. The range in quelea preference-response varied from slight indifference to valonea extract (mean preference score $>$ 40%), to rejection of quebracho, chestnut, myrabolam, and wattle extracts (mean preference score $<$ 40%, Table 1). No overall differences in response were attributable to the test compounds (ANOVA, $p > 0.05$).

TABLE 1. Quelea preference response to respective 0.2% vegetable tannin-coated vs control millet seeds (individual cage tests, six birds per treatment).

Source of tannin (extract)	Consumption (g) Mean ± SD		P (t-test)	Percent preference[1] Mean ± SD[2]
	Control food	Treated food		
Valonea	13.0 ± 2.5	8.7 ± 2.2	>0.05	40.8 ± 10.3
Quebracho	17.8 ± 3.6	4.1 ± 1.8	<0.01	18.8 ± 7.3
Chestnut	15.3 ± 5.3	5.3 ± 2.8	<0.05	28.5 ± 9.8
Wattle	15.3 ± 3.1	6.0 ± 3.2	<0.02	27.9 ± 10.7
Myrabolam	15.8 ± 2.5	7.0 ± 3.5	<0.02	32.5 ± 10.9

[1]The percent preference is the percent by weight that the treated food made up of the total food consumed (treated food consumed + control food = 100 percent).

[2]Not significantly different from each other ($P > 0.05$, one-way ANOVA).

However, observation of results within each test permitted a closer look at each tannin product. The standard deviation and paired *t*-tests reflect some differences in response patterns. This is typical of tannins, since they are known to occur in plant extracts as polyphenols of varying molecular structure, size, and complexity. Similar results were obtained on quelea groups in enclosures (Bullard and Shumake, 1979).

The chemical characteristics and astringent properties of wattle (*Acacia mearnsii*) tannins are well known (Roux, 1972). This consideration, in addition to the performance of wattle tannin in this preference test, favored the selection of wattle extract for further investigation of its repellent characteristics. More pertinent to the objective of this study (development of an inexpensive and nontoxic bird repellent) is the fact that the wattle bark extract industry is native to Africa, which indicates the commercial availability of the product at low cost.

Test 2 · Optimum Dose Determination

This test was conducted to establish an intermediate dosage level for subsequent tests. Levels too high or too low would not be useful in measuring tannin differences. Test 1 results and previous DWRC experience with commercial tannins (Bullard and Shumake, 1979) provided the basis for selecting wattle tannin and four treatment levels (0.1, 0.15, 0.2, and 0.25%).

The preference means (Table 2) for birds tested at 0.15, 0.2 and 0.25% levels ranged within the rejection zone ($<$40%), whereas birds tested at the 0.1% level showed indifferent responses to the treatment ($>$40%). A one-way analysis of variance indicated an overall difference in the preference response as a function of the dosage level ($P < 0.05$). The preference for the treatment at 0.2 and 0.25% levels was significantly less than that at 0.1 and 0.15% levels ($P < 0.05$). Individual tests analyzed by paired *t*-test indicated that at the 0.1% level there were no significant differences between treated and control foods ($P > 0.05$). As the dosage level increased, wattle tannin elicited a repellent effect ($P < 0.05$). Overall, the wattle-tannin treatment threshold (the lowest concentration at which the consumption of treated food differed significantly from that of control food) seemed to be at about 0.15% concentration.

The large standard deviations indicated considerable variation among birds, both in total food intake and preference. Kare and Ficken (1963) attributed this phenomenon to differences in sensitivity and gross anatomy of the taste system among individuals. As in our studies, they observed a consistent pattern of increasing rejection and decreasing individual variation in response with increasing concentration. The intermediate percent preference value (35%) observed for 0.2% wattle tannin indicated that this level met our objective.

TABLE 2. Quelea preference response to respective level of wattle-tannin treated vs control millet seeds (individual cage tests, six birds per treatment).

Tannin concentration %	Consumption (g) Mean ± SD		P (t-test)	Percent preference[1] Mean ± SD[2]
	Control food	Treated food		
0.1	11.9 ± 3.4	8.4 ± 3.4	>0.05	40.4 ± 11.2[a]
0.15	14.3 ± 3.7	8.6 ± 1.9	<0.05	38.2 ± 10.9[a]
0.2	13.2 ± 1.6	7.2 ± 1.4	<0.001	35.0 ± 3.5[a,b]
0.25	17.8 ± 3.4	5.7 ± 2.6	<0.01	24.3 ± 10.9[b]

[1]The percent preference is the percent by weight that the treated food made up of the total food consumed (treated food consumed + control food consumed = 100 percent).

[2]Means followed by the same superscript letter are not significantly different from each other by Duncan's New Multiple Range Test ($P < 0.05$).

Test 3 · Extraction Enhancement of Tannin Activity

Since condensed tannin oligomers of intermediate molecular weight are more astringent than high molecular weight polymers (Bullard and Elias, 1980), we attempted to enhance tannin activity through extraction. Following the procedure of Roux and Paulis (1961), wattle tannin was fractionated into two molecular weight classes.

This was accomplished as follows: two 25 g samples of commercial wattle extract were placed in 250-ml centrifuge bottles. Each sample was then extracted by shaking for 30 minutes with 100 ml absolute methanol. The supernatants from each bottle were combined in a 1-l flask, concentrated by rotary evaporation, and brought to dryness overnight in a vacuum oven set at 10° C and 20 lb/inch^2. Unextractable residues were combined and air dried. Unfractioned wattle, wattle fraction extractable with absolute methanol (WF1), and that fraction not extractable with the same solvent (WF2) were then individually tested for repellency at 0.2% w/w on hulled proso millet.

The percent preference scores measured for the treatments ranged from 13.1 to 39.9 (Table 3). Significant differences in quelea food preference response as a function of wattle tannin components were observed ($P < 0.01$). Food consumption patterns of the three wattle-tannin treatments indicated similar repellent responses that differed quantitatively. The preference scores for unfractioned wattle (22%) and WF1 (13.1%) were significantly less than the value observed for WF2 (39.3%) but not essentially different from each other. Paired *t*-test analysis of individual preference tests indicated consumption differences between treatment and control for unfractionated wattle and WF1 but not for WF2 ($P > 0.05$).

Intake rates of both the control and treated food varied among individual birds (Fig. 1). This variability probably is dependent on whether a bird was sensitive (responder) or refractory (non-responder) to the treatment. Whereas a responder avoided the treated food and fed almost exclusively on the reference food, a non-responder sampled both foods. This effect occurs more often with unfractionated wattle at this concentration. Either the lower concentration of protein-binding molecules produces a slower astringent response in the mouth or the threshold itself is barely met.

TABLE 3. Quelea preference response to wattle tannin and its two fractions coated at 0.2% on millet vs untreated millet (individual cage tests, six birds per treatment).

Formulation	Percent preference[1] Mean ± SD[2]	*P*(one-way ANOVA)	Consumption (g) Mean ± SD Control food	Consumption (g) Mean ± SD Treated food	*P* (t-test)
Wattle unfractioned	22.0 ± 17.5^b		14.9 ± 4.1	4.2 ± 3.6	<0.02
WF1[3]	13.1 ± 4.6^b	<0.01	17.9 ± 1.4	2.7 ± 1.2	<0.001
WF2[3]	39.3 ± 10.4^a		10.7 ± 1.9	6.9 ± 1.9	>0.05

[1]The percent preference is the percent by weight that the treated food made up of the total food consumed (treated food consumed + control food = 100 percent).

[2]Means followed by the same superscript letter are not significantly different from each other by Duncan's New Multiple Range Test ($P<0.05$).

[3]WF1 and WF2 are arbitrary designations for wattle molecular components extractable with absolute methanol and those not extractable with the same solvent, respectively.

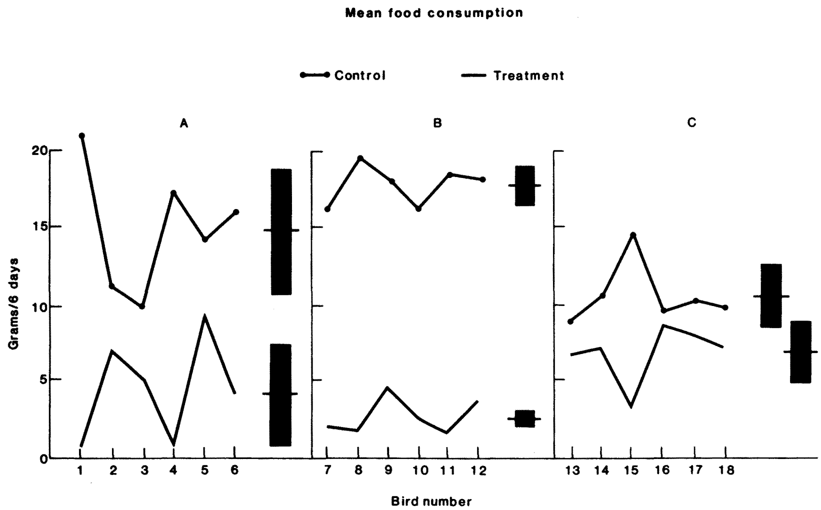

FIGURE 1. Food consumption measured for three groups of quelea in a series of two-choice preference tests. Each group of six birds was presented with a control and treated food daily for six days. The treatments were: 0.2% unfractioned wattle (A), 0.2% wattle fraction extractable with absolute methanol (B), and 0.2% wattle fraction unextractable with the same solvent (C). Means corresponding to control and treatments are indicated with horizontal lines. Rectangles indicate SD on each side of the six-day means.

The absolute methanol fraction (WF1) consisted mainly of oligomeric flavans (flavan units of intermediate molecular size) that are optimal protein-binders because of their size (Goldstein and Swain, 1963). Conversely, the low repellent activity observed for the WF2 fraction is attributable to the predominance of high molecular weight polymer. The fact that unfractionated wattle was not significantly different in preference from WF1 indicated that the small advantage in increased protein binding activity is not worth the cost of extraction. Overall activity of the repellent formulation could be increased by simply adding more of the inexpensive wattle tannin.

Test 4 - Enclosure Evaluation of Wattle Tannin Repellency

This test was designed to determine the preference response of quelea to the highest practical field application rate (dipping in 0.5% wattle tannin solution ~ 15 kg/ha) under simulated field conditions.

Paired *t*-tests indicated there were no significant differences between treated and control food consumption in any of the three tests. From a surface-coating standpoint, the effective treatment level for this test was about 0.1% w/w which in Test 1 of the cage tests also failed to elicit a repellent response. Thus, wattle tannin applied topically at a high application rate (~ 15 kg/ha) did not repel birds; because of the impracticality of going to higher levels, it was not considered to be a likely candidate for further bird repellent tests at the Denver Wildlife Research Center.

TABLE 4. Feeding response of quelea (N = 10) to sorghum heads (control and dipped in 0.5% wattle-tannin solution) presented in an enclosure test.

Replication	Percent preference[1]	*P*(t-test)
1 Aug. 1978[2]	42.0	>0.1
2 Nov. 1979	51.3	>0.1
3 Sept. 1980	50.6	>0.1

[1]The percent preference is the percent by weight that the treated food made up of the total food consumed (treated food consumed + control food = 100 percent).

[2]Bullard and Shumake, 1979.

SUMMARY

This study was conducted to investigate and assess the potential of commercial wood tannins as repellents for birds in topical applications to cereal grains. The following results were obtained:

1. Four extracts (quebracho, chestnut, wattle, and myrabolam) of the five commercial tannin preparations tested possessed repellent potential (mean percent preference scores were less than 40).
2. Wattle tannin was selected as a candidate repellent because of its observed repellent activity, economic considerations, and the greater knowledge available on its chemical characteristics.
3. A structure-function relationship was demonstrated for wattle tannin whereby the oligomeric fraction ranked higher than unfractionated wattle. However, there were no significant differences between the two, and the costs incurred in a similar extraction process would not make this step cost-effective.
4. Wattle tannin levels of 15 kg/ha did not protect sorghum heads from quelea depredation under group enclosure test conditions.

Under these conditions, it would not seem that commercial wood tannins would be suitable repellents to use in protecting cereal crops from quelea depredation. Nevertheless, in subsequent tests wattle tannin effectively enhanced the repellent properties of methiocarb formulations (Bullard et al., 1983).

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